Induction of Plasminogen Activator Inhibitor by Products Released From Platelets

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Activation of platelets and augmentation of plasma plasminogen activator inhibitor (PAI) type I activity accompany acute myocardial infarction. To determine whether the two may be related, platelet compounds including epidermal growth factor and transforming growth factor β as well as platelet lysates were studied in rabbits in vivo. After intravenous infusion of epidermal growth factor (1 and 5 μg/kg), plasma PAI activity increased sevenfold and 20-fold, peaking at 2 hours. After infusions of transforming growth factor β (0.2 and 0.5 μg/kg), plasma PAI activity increased sevenfold and 12-fold but peaked more slowly (at 5 hours). After infusion of platelet lysates (lysates from 2.8 and 5.6×10⁸ platelets/kg), the increase was 19-fold and 35-fold, with a peak at 4 hours. Platelet lysates induced a pronounced increase of plasma PAI type 1 messenger RNA (Northern blots) in aorta, liver, and myocardium. Anti–transforming growth factor β neutralizing antibody markedly attenuated the plasma PAI increase. Concentrations in plasma of fibrinogen and α₂-antiplasmin were virtually unaffected under all conditions. Thus, platelet-associated growth factors and platelet lysates, shown previously to increase plasma PAI type 1 messenger RNA expression and protein production in cultured hepatocytes and vascular endothelial cells in vitro, augment plasma PAI in vivo as well. Accordingly, activation of platelets and release of platelet-associated growth factors appear to contribute to the increased plasma PAI seen after myocardial infarction. (Circulation 1990;82:1485–1493)

The efficacy of pharmacological coronary thrombolysis appears to depend on the relative intensity of fibrinolysis and thrombosis in the immediate vicinity of targeted thrombi.1–4 Local fibrinolysis, mediated in part by plasminogen activators elaborated from endothelium as well as activator administered pharmacologically, may be attenuated by inhibitors including α₂-antiplasmin and plasma plasminogen activator inhibitor (PAI) type 1 (PAI-1).5,6 Increases in plasma PAI activity have been implicated as a risk factor for myocardial infarction.7,8

We recently found that plasma PAI-1 messenger RNA (mRNA) levels and protein synthesis9,10 in cultured human hepatocytes are augmented by platelet-associated growth factors including epidermal growth factor (EGF) and transforming growth factor β (TGF-β), and by crude platelet lysates. Others have reported increases in PAI-1 mRNA in cultured bovine aortic endothelial cells.11 In view of the high concentrations of platelet products within thrombi (approximately 200-fold greater than the concentrations in whole blood12), we have hypothesized that platelet products may attenuate fibrinolysis in vivo by augmenting endothelial cell synthesis and release of plasma PAI locally in the vicinity of thrombi, and hepatic elaboration of PAI-1 into the circulation.1 Such attenuation could potentially be modified with the use of plasma PAI-1 invisible mutants of plasminogen activators13 and by conjoint use of antiplatelet and fibrinolytic drugs. The present study was performed to determine whether our previous observations with hepatocytes in vitro were applicable in rabbits in vivo and to determine whether platelet lysates induced increased concentrations of plasma PAI. Additionally, it addressed mechanisms by which platelet activation may affect PAI concentrations in plasma in vivo in studies using antibodies to specific growth factors released.

Methods

Materials

EGF (from mouse submaxillary gland), rabbit anti-EGF IgG, and TGF-β (from human platelets) were purchased from Collaborative Research (Bedford, Mass.), rabbit anti–TGF-β IgG and [¹²⁵I]TGF-β
(118.8 μCi/μg 91% trichloroacetic acid [TCA] precipitable) from R&D Systems (Minneapolis, Minn.), [125I]EGF (100 μCi/μg 95% TCA precipitable) and [32P]deoxycytidine 5’-triphosphate (dCTP) from Amersham (Arlington Heights, Ill.), D-phenylalanyl-l-propyl-l-arginyl-chloromethyl ketone (PPACK) from Calbiochem (San Diego, Calif.), human Glu-plasminogen and synthetic chromogenic substrate D-Val-Leu-Lys-pNA (S-2251) from Bio-Rad (Richmond, Calif.), and molecular weight standards from Bio-Rad (Richmond, Calif.), restriction endonucleases from New England Biolabs (Beverly, Mass.), and chemical reagents of highest available grade from Sigma Chemical (St. Louis, Mo.).

**Determination of Tissue-Type Plasminogen Activator Activity**

Tissue-type plasminogen activator (t-PA) activity was assayed in citrated plasma with a modification of the spectrophotometric assay developed by Angles-Cano et al. as described previously. Plasma samples were incubated in microtiter wells coated previously with fibrin monomer for 2 hours at 37°C. t-PA activity was determined with 0.2 mM Glu-plasminogen and 1.0 mM S-2251 over 2 hours at 37°C and calibrated with respect to concomitantly assayed standards (International Reference Preparation of t-PA (83/517) obtained from the National Institute for Biological Standards and Control [London]) in pooled plasma.

**Determination of PAI Activity and the Concentration of PAI-1 Antigen**

PAI activity was assayed spectrophotometrically in citrated plasma with a modification of the assay developed by Chmielewska and Wiman as described previously. One arbitrary unit (AU) of plasma PAI was defined as the amount of activity that inhibited one international unit (IU) of t-PA over 10 minutes. Standard curves were obtained with serial dilutions of pooled rabbit plasma, and results were expressed as arbitrary units per milliliter (AU/ml). The interassay coefficient of variation was 4.3%.

Concentrations of PAI-1 antigen were determined as described by Declerck et al. with reagents obtained from American Diagnostica (New York).

**Other Biochemical Procedures**

Fibrinogen was assayed by the sulfite precipitation method, and α2-antiplasmin was assayed spectrophotometrically as previously described. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) (4% stacking gel and 7.5% separating gel) was performed conventionally with Laemml buffers. Plasminogen activator and PAI activity in rabbit plasma were detected by SDS-PAGE fibrin autogrophy and by reverse fibrin autogrophy, respectively.

**Preparation of Platelet Lysates**

Platelets were isolated from rabbit platelet-rich plasma as previously described. Lysates were prepared by freezing in liquid nitrogen and thawing in a 37°C water bath three times. Supernatant fractions were stored at −70°C for 1 week and thawed once for infusion of platelet lysates intravenously in the same platelet-donor rabbit. Lysate obtained from 10⁶ platelets inhibited 0.53±0.07 IU/ml t-PA (mean ± SD, n=6). Inhibitory effects were linearly related to platelet lysate concentration. The absence of endotoxin in platelet lysate preparations was confirmed with the limulus amoebocyte lysate assay (Pyrotell, Associates of Cape Cod, Woods Hole, Massachusetts).

**Procedures in Experimental Animals**

Care and handling of experimental animals conformed to the standards established by the Washington University Committee for Humane Care of Laboratory Animals consistent with those of the Helsinki declaration. New Zealand White rabbits (2.5–3.3 kg body wt) were supplied by the Boswell Bunny Farm (St. Louis, Missouri). Rabbits were anesthetized with intramuscular injection of 0.1 ml of Inovar-Vet (0.4 mg/ml fentanyl and 20 mg/ml droperidol) and ketamine (20 mg/kg) in dosages sufficient to maintain analgesia and anesthesia. Subsequently, intravenous bolus injections of pentobarbital were administered as needed to maintain adequate analgesia and anesthesia. Growth factors and platelet lysates were administered through an external jugular vein catheter. For acquisition of plasma samples, whole blood was collected from an external jugular vein or femoral artery catheter with a two-syringe technique in plastic syringes and transferred immediately into tubes maintained at 0–4°C containing sodium citrate (12.9 mM final concentration) as previously described. Catheters were flushed with 3 ml sterile saline after acquisition of each sample. Plasma samples were separated quickly by centrifugation at 4°C at 2,000g for 15 minutes, aliquots were frozen immediately in liquid nitrogen, and samples were stored at −70°C for subsequent assay. Samples for assay of fibrinogen were collected in citrate tubes with PPACK (2 μM). All assays were performed within 1 week. Blood leukocyte and platelet counts were determined conventionally with a hemocytometer.

**Pharmacokinetic Studies**

Rabbits were given [125I]EGF (6.1 ng/kg) or [125I]TGFB-β (4.9 ng/kg) in 0.5-ml aliquots of phosphate-buffered saline by intravenous bolus injection through the femoral vein. All rabbits were pretreated with 100 mg NaI injected intraperitoneally 10 minutes before injection of growth factor to block thyroidal uptake of released radioactive iodide. Serial blood samples were obtained at selected intervals by the femoral artery into sodium citrate tubes and centrifuged to yield plasma samples (500 μl) that were supplemented with trichloroacetic acid (20%
wt/vol) for precipitation of protein at 0–4°C. Precipitates were assayed for $^{125}$I radioactivity in a gamma well counter to provide estimates of the concentration of each of the growth factors in plasma as a function of time after injection. Semilog plots were constructed for calculation of distribution and elimination rate constants, and half-lives, with a Perkin-Elmer 3230 computer as previously described.23

Organ Distribution of Injected Growth Factors

In separate experiments, rabbits were injected with $^{[125]}$IEGF (6.1 ng/kg) or TGF-β (4.9 ng/kg). Fifteen minutes after injection of EGF and 30 minutes after injection of TGF-β, the rabbits were given overdoses of pentobarbital. Organs were harvested rapidly in random sequence, washed extensively twice in ice-cold phosphate-buffered saline, blotted on filter paper, minced with a razor blade, and weighted before determination of $^{125}$I radioactivity in a gamma well counter. Concentrations of each growth factor in individual organs were determined from averages of radioactivity per gram of tissue and specific activators of the injected growth factor in three different rabbits. Blood was obtained immediately before organs were harvested, and plasma was separated and saved for further analysis.

Preparation of Complementary DNA Probes, Isolation of RNA, and Assay of PAI-1 Messenger RNA

Rabbits exposed to exogenous platelet lysates were given overdoses of pentobarbital at selected intervals, and organs were removed rapidly, rinsed twice in phosphate-buffered saline solution at 0–4°C, frozen quickly in liquid nitrogen, and stored at −70°C. Results with each intervention were based on assay of organs from at least three rabbits.

A 1.1 kb PAI-1 complementary DNA (cDNA) probe for PAI-1 mRNA was generated by digestion of PAI-1 cDNA provided by T.C. Wun (Monsanto, St. Louis, Missouri)24 with EcoRI and Ava I. The probe was isolated by batch affinity adsorption with sodium iodide glass beads (GENECLEAN, Bio 101, La Jolla, California).

A probe for glyceraldehyde-3-phosphate dehydrogenase mRNA (GAP, 0.6 kb) was obtained by Xba/HindIII digestion of cDNA (457091, American Type Culture Collection, Rockville, Md.).25 Its integrity was verified by ethidium bromide visualization after agarose gel electrophoresis.

cDNA probes were labeled with $^{[32]}$PdCTP by the random primer technique according to the manufacturer’s recommended procedure (Boehringer Mannheim, Indianapolis, Indiana)26 to a specific activity of 1 x $10^7$ dpm/μg. Total RNA was extracted with the proteinase K technique.27 The integrity of isolated RNA was verified by ethidium bromide staining.28

Total RNA (2.5 μg) in each sample was quantified based on absorbance at 260 nm and separated in 1.5% formaldehyde agarose gels. Northern blotting was performed by capillary transfer to nylon membranes (GeneScreen, DuPont–New England Nuclear, Wilmington, Delaware). Blots were baked at 80°C for 2 hours in a vacuum, and hybridization was performed conventionally.9 Briefly, membranes were prehybridized in a solution of 50% deionized formamide, x10 Denhardt’s solution, 0.05 M Tris-HCl, 1.0 M NaCl, 0.1% sodium pyrophosphate, 1% SDS, 10% dextran sulfate, and 100 μg/ml sonicated denatured salmon sperm DNA for at least 6 hours at 42°C with agitation.

Hybridization with 320,000 dpm/ml PAI-1 and 160,000 dpm/ml GAP of labeled probe was performed at 42°C for 20–24 hours. After hybridization, membranes were washed three times for 20 minutes each at 55°C with 1% SDS and 2x, 1x, and 0.5x SSC (1x SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), respectively. Blots were assayed with a radioisotopic scanner (Ambis Radioisotope Scanner, Automated Microbiology System, San Diego, California) for quantification of radioactivity corresponding to each band. Hybridization to the GAP probe was used as an internal control to ensure consistent quantity and integrity of RNA samples present on the blot after transfer and hybridization.

 Autoradiography was performed with Kodak XAR-5 film and intensifying screens (Cronex lightning plus, DuPont–New England Nuclear) at −70°C. The size, in kilobases, of detected mRNA species was calculated on the basis of migration of DNA standards from the gel wells.

Statistical Analysis

Results are presented as mean±SD of the mean. Repeated measures analysis of variance was used to assess changes in physiological variables over time. A p value less than or equal to 0.05 was considered indicative of a significant difference.

Results

Effect of Growth Factors and Platelet Lysates on Plasma PAI-1 Activity In Vivo

An early increase in plasma PAI activity (p<0.05) was evident within 1 hour in rabbits given EGF (Figure 1, upper left panel), with a peak in 2 hours and subsequent rapid decline. The plasma PAI levels were significantly elevated above baseline up to 4 hours after administration in the group of rabbits receiving 1 μg/kg and up to 6 hours in the group of rabbits receiving 5 μg/kg. Increases were greater with 5 μg/kg than with 1 μg/kg doses of EGF. When EGF (1 μg/kg) was neutralized with anti-EGF antibody before injection, no significant changes in plasma PAI activity ensued.

In rabbits given TGF-β (0.2 or 0.5 μg/kg), PAI activity in plasma was significantly increased after 2 hours. In comparison with the response to EGF, however, the time course of the increase differed, with a peak in 5 hours and a gradual decline subsequently (Figure 1, upper right panel). The plasma PAI levels were significantly elevated above baseline up to 6 hours after administration in the groups of rabbits receiving both 0.2 and 0.5 μg/kg.
There was no significant difference in plasma PAI response in the groups of rabbits receiving either 0.2 or 0.5 μg/kg.

To determine whether platelet lysates increased PAI activity in plasma, autologous platelet lysates prepared 1 week before the experiment were given intravenously to rabbits (lysates from 2.8 or 5.6×10^8 platelets/kg body wt). Plasma PAI activity increased significantly from 2 to 6 hours after administration in the group of rabbits receiving 2.8×10^8/kg and from 1 to 6 hours in the group of rabbits receiving 5.6×10^8/kg. The peak effect was observed at 3–4 hours. Activity remained elevated to 45–55% of peak activity after 6 hours (Figure 1, lower panel). Increases were significantly greater with the more concentrated lysates. Infusion of platelet-poor plasma had no significant effect on plasma PAI activity. When platelet lysates were pretreated with anti-TGF-β neutralizing antibody, plasma PAI levels were still significantly elevated above baseline from 2 to 6 hours after administration. Stimulatory effect of lysates on plasma PAI activity, however, were markedly attenuated. Thus, after 2 hours, the magnitude and duration of plasma PAI increase was significantly reduced compared with values in rabbits given lysates that had not been neutralized with antibody to TGF-β (Figure 1, lower panel). Accordingly, one of the components in lysates responsible for augmentation of plasma PAI activity appeared to be TGF-β. In contrast, when 2.8×10^8/kg platelet lysates were pretreated with anti-EGF neutralizing antibody, the initial increases of PAI activity in plasma were not attenuated at all (n=2, data not shown).

Assays of plasma PAI-1 antigen concentrations with an enzyme-linked immunosorbent assay (ELISA) developed for human plasma PAI-1 correlated closely with plasma PAI activity (r=0.94, n=56). The increases of plasma PAI activity were demonstrable also by reverse fibrin autography after SDS-PAGE of plasma samples (data not shown).
Total blood leukocyte counts did not increase significantly over 24 hours in groups of rabbits treated with platelet lysate (Table 1). Platelet counts remained constant after injection of EGF, TGF-β, or platelet lysates (Table 1). Thus, the increased PAI-1 in plasma was not likely to come from activation of circulating platelets.

To determine whether the increased PAI activity in plasma may have been a manifestation simply of an acute phase reaction, α2-antiplasmin and fibrinogen were assayed before (baseline) and at the time of the observed peak plasma PAI activity, and 24 hours after administration of growth factors or platelet lysates. Changes in these acute phase reactants were undetectable or trivial (Table 2). α2-Antiplasmin and fibrinogen levels decreased slightly at 2 or 3 hours, followed by only a slight increase at 24 hours (Table 2) with no dose-response relation to the concentrations of lysates.

Another acute phase reactant is functional t-PA activity, which increases transiently with a peak approximately 1 hour after infusion of endotoxin in humans or rabbits. Accordingly, t-PA activity was measured in rabbit samples. No early increase in t-PA activity was evident after administration of growth factors or platelet lysates, as judged from results of spectrophotometric assays or fibrin autography, which would have reflected increases even in the presence of increased plasma PAI-1. These results are consistent with the absence of a generalized, acute phase reaction.

**Pharmacokinetics and Organ Distribution of Injected Growth Factors**

Plots of radiolabeled [125I]EGF and [125I]TGF-β concentrations in plasma as a function of time after injection were consistent with first-order kinetics after rapid distribution (Figure 2). In the distribution phase after intravenous bolus injections, both growth

### Table 1. Leukocyte and Platelet Counts at Selected Intervals After Injection

<table>
<thead>
<tr>
<th>Injected material</th>
<th>Leukocytes/mm³ (×10⁻⁹)</th>
<th>Platelets/mm³ (×10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Hr</td>
<td>2 Hr</td>
</tr>
<tr>
<td>EGF 1 μg/kg (n=3)</td>
<td>51±9</td>
<td>55±4</td>
</tr>
<tr>
<td>5 μg/kg (n=3)</td>
<td>62±11</td>
<td>66±4</td>
</tr>
<tr>
<td>TGF-β 0.2 μg/kg (n=4)</td>
<td>66±19</td>
<td>56±10</td>
</tr>
<tr>
<td>0.5 μg/kg (n=4)</td>
<td>60±17</td>
<td>64±17</td>
</tr>
<tr>
<td>Platelet lysate 2.8×10⁶ platelets/kg (n=4)</td>
<td>57±6</td>
<td>73±32</td>
</tr>
<tr>
<td>5.6×10⁶ platelets/kg (n=4)</td>
<td>51±11</td>
<td>58±22</td>
</tr>
</tbody>
</table>

Results expressed as mean±SD. EGF, epidermal growth factor; TGF-β, transforming growth factor β.

### Table 2. α2-Antiplasmin and Fibrinogen (% Control)

<table>
<thead>
<tr>
<th>Injected material</th>
<th>α2-Antiplasmin</th>
<th>Fibrinogen</th>
<th>α2-Antiplasmin</th>
<th>Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Hr</td>
<td>24 Hr</td>
<td>2 Hr</td>
<td>24 Hr</td>
</tr>
<tr>
<td>EGF 1 μg/kg (n=3)</td>
<td>96±3</td>
<td>117±10*</td>
<td>85±30</td>
<td>114±9</td>
</tr>
<tr>
<td>5 μg/kg (n=3)</td>
<td>103±7</td>
<td>132±26</td>
<td>117±20</td>
<td>149±26*</td>
</tr>
<tr>
<td>TGF-β 0.2 μg/kg (n=4)</td>
<td>94±6</td>
<td>104±22</td>
<td>105±15</td>
<td>147±21*</td>
</tr>
<tr>
<td>0.5 μg/kg (n=4)</td>
<td>99±8</td>
<td>118±14</td>
<td>98±6</td>
<td>131±18*</td>
</tr>
<tr>
<td>Platelet lysate 2.8×10⁶ platelets/kg (n=4)</td>
<td>92±7</td>
<td>112±10*</td>
<td>92±11</td>
<td>138±14*</td>
</tr>
<tr>
<td>5.6×10⁶ platelets/kg (n=4)</td>
<td>95±10</td>
<td>136±40</td>
<td>91±17</td>
<td>136±16*</td>
</tr>
</tbody>
</table>

Values are expressed as percentage of value before injections (mean±SD).

*p<0.05, compared with values before injections.
Factors were cleared rapidly from the circulation with a half-life of 0.8 (EGF, n=3) and 1.1 minutes (TGF-β, n=7). The β half-lives of clearance were 48.4 (EGF) and 37.2 minutes (TGF-β).

High concentrations of [125I]-labeled EGF and TGF-β after intravenous bolus injection were found in kidney, liver, spleen (Figure 2), and lung, in which the concentration of TGF-β was maximal. Concentrations in these organs were 20–70-fold greater than in blood (radioactivity/g). Thus, tissue concentrations could not be explained simply by trapping of blood, especially after the extensive rinsing used. Tissues demonstrating the lowest uptake of labeled protein included skeletal muscle (1.3% of values in organs with the highest concentrations). Uptake was intermediate in heart and lung (EGF), and aorta (TGF-β).

Whole organ distribution patterns were calculated based on organ weights and total blood volumes. Liver and kidney accumulated approximately 90% of the total injected dose of EGF (Figure 3). Liver, lung, and kidney accumulated approximately 70% of the total injected doses of TGF-β (Figure 3).

To verify the lack of degradation of injected labeled TGF-β, plasma obtained 5 minutes after

**Figure 2.** Graphic plots (insets) of pharmacokinetics of radiolabeled epidermal growth factor (EGF) (upper panel) and transforming growth factor β (TGF-β) (lower panel) after intravenous bolus injection. [125I]EGF and [125I]TGF-β radioactivity (cpm) in serial 500-μl blood samples obtained at selected intervals after intravenous bolus injection of [125I]EGF (n=3) and [125I]TGF-β (n=7) are shown (mean±SD). Elimination half-lives were calculated as described in the “Methods” section. Organ distribution of [125I]EGF (n=5) and [125I]TGF-β (n=4) after intravenous bolus injections. Histograms show [125I]EGF and [125I]TGF-β concentrations (cpm/g) (mean±SD) in selected organs.

**Figure 3.** Histograms show the total accumulation of [125I]-labeled epidermal growth factor (EGF) (n=5) (left panel) and [125I]-labeled transforming growth factor β (TGF-β) (n=4) (mean±SD) (right panel) in selected organs 15 and 30 minutes after injection.
injection of the growth factor was assayed by autoradiography after SDS-PAGE. Only intact, radiolabeled TGF-β polypeptide was evident. Similarly, autoradiographic analysis of liver and heart homogenates harvested 30 minutes after injection of [125I]TGF-β demonstrated a single band corresponding to intact TGF-β.

Figure 4. Photomicrographs of plasminogen activator inhibitor type I (PAI-1) messenger RNA (mRNA) in aorta, liver, and heart. Representative Northern blots of RNA from rabbit aorta, liver, and heart RNA from control rabbits and rabbits given platelet lysates intravenously are shown. Total RNA from each tissue (2.5 μg) was fractionated by formaldehyde agarose gel electrophoresis, transferred to nylon membrane, hybridized with [32P]PAI-1 complementary DNA (cDNA), and assayed by autoradiography. In rabbits given platelet lysates, tissues were harvested 3 hours after infusion of lysates of 5.6×10⁷ platelets/kg body wt. A representative blot typical of results in each of 3 experiments is shown. Glyceraldehyde-3-phosphate dehydrogenase (GAP) mRNA (control) is shown at the bottom.

Effect of Platelet Lysates on Expression of PAI-1 Messenger RNA in Rabbit Tissues In Vivo

As previously reported, PAI-1 mRNA in human tissues appears as 3.2 and 2.2 kb species in different proportions typical of different tissues. In the present studies, however, only one PAI-1 mRNA species (3.2 kb) was detectable in extracts from rabbit tissues even with replicated Northern blots subjected to stringent conditions.

In rabbits given platelet lysates, PAI-1 mRNA levels were increased (three- to fivefold) in aorta and heart as well as in liver (Figure 4) (n=3). No increases were seen in lung, kidney, spleen, or muscle. The apparent concentration (control) of GAP mRNA did not change (Figure 4).

Discussion

Results of this study indicate that platelet lysates increase PAI activity in rabbit plasma in vivo and PAI-1 mRNA in aorta, liver, and heart. Additionally, platelet lysates augment plasma PAI-1 protein in conditioned media and PAI-1 mRNA in cultured cells. Thus, it appears likely that platelet activation associated with phenomena such as coronary thrombosis and coronary thrombolysis in vivo may augment plasma PAI-1 locally, thereby attenuating fibrinolysis mediated by endogenous plasminogen activators and exogenous agents such as t-PA. The increases in plasma PAI activity in vivo were seen with plasma concentrations of EGF, TGF-β, and platelet lysates that did not markedly influence concentrations of α₂-antiplasmin or fibrinogen, and that were consistent with physiological levels and those anticipated after myocardial infarction. Thus, although plasma PAI-1 increases rapidly after surgery or trauma, the changes we observed cannot be explained simply in terms of an acute phase reaction.

PAI in plasma appears to be identical to that produced by endothelial cells. The increased plasma PAI activity we observed, paralleled by an increase in plasma PAI-1 antigen, could reflect release of PAI from platelets increased release from endothelium, hepatocytes, or other tissue, or both. Release of PAI-1 from platelets was probably pivotal because 1) platelet counts did not decline, 2) platelets in citrated platelet-rich plasma did not aggregate in response to EGF (up to 100 ng/ml) or TGF-β (up to 100 ng/ml) (unpublished observations), 3) high concentrations of thrombin capable of inducing more than a 50% decline in platelet counts do not augment PAI activity in plasma, and 4) much of the inhibitor present in platelets may be inactive or latent.

As judged from the changes in concentrations of PAI-1 mRNA in tissues, it appears likely that endothelial cells and hepatocytes are responsible for the increases in PAI activity in plasma induced by platelet lysates in vivo. This hypothesis is consistent with our observations in cultured cells demonstrating increased elaboration of PAI-1 in response to platelet lysates in association with an increase in steady-state levels of PAI-1 mRNA.

Activity of growth factors used in the present study is present in human platelets. Concentrations studied are consistent with those attributable to release of growth factors from platelets at sites of thrombolysis in vivo, based on estimates of mean concentrations of PAI-1 mRNA in aorta, liver, and heart.
antiplatelet volume and estimates of concentrations of platelets in typical platelet plugs.

Both EGF and TGF-β increased PAI activity in plasma in vivo in a concentration-dependent fashion but with diverse time courses. It appears likely that the effects of platelet lysates on plasma PAI were mediated by at least two platelet products because neutralizing antibody to TGF-β failed to inhibit the early increase of plasma PAI activity characteristic of that seen with EGF. Antibody to EGF, which blocked the EGF-induced plasma PAI increase, however, failed to inhibit the increase of plasma PAI induced by lysates. It is possible that the anti-EGF antibody we used might have failed to detect the EGF receptor–binding peptide in platelets, which may be a TGF-α molecule that binds to the same receptor as EGF.

The relatively gradual increase of PAI in plasma in vivo may reflect participation of de novo protein synthesis in the process. This hypothesis is supported by our observations of increased PAI-1 mRNA induced by platelet lysates in rabbit tissues. Some of the PAI-1 secreted from cells appears to accumulate in extracellular matrix before subsequent release. The increases of PAI-1 mRNA we observed in rabbit tissues, however, are consistent with an effect of platelet lysates on synthesis of PAI-1 protein per se.

EGF and TGF-β accumulated to a considerable extent in liver. Human hepatoma (Hep G2) cells in culture exposed to TGF-β for only 30 minutes exhibit an increase in PAI-1 mRNA. Even brief platelet activation may release TGF-β, shown in this study to be cleared with a half-life of approximately 30 minutes, in quantities sufficient to increase PAI-1 mRNA in hepatocytes. The activity of PAI in human platelets is an order of magnitude greater than that in rabbit platelets. Accordingly, in fresh human thrombi, the concentration of PAI may be even higher because of release from platelets participating in clotting. The presence of platelet-derived PAI and the effects of factors from platelets leading to increased PAI synthesis may both contribute to resistance of platelet-rich thrombi to plasminogen activators.

The present results are consistent with the hypothesis that platelet-released growth factors may augment PAI-1 concentrations locally and in the circulation, thereby attenuating coronary thrombolysis and potentially limiting its efficacy by retarding recanalization or predisposing to early reclosure. Accumulation of platelets at sites of endothelial injury and their consequent release of growth factors may explain the high concentrations of TGF-β found in coronary thrombi. These phenomena are particularly promising targets for adjunctive pharmacological approaches to potentiation of coronary thrombolysis with antiplatelet drugs, antibodies, or neutralizing F(ab′)2 fragments directed against circulating or locally released growth factors, and inhibitor-invisible plasminogen activators. Our results are consistent also with the possibility that some of the beneficial effects of antiplatelet agents in patients treated with plasminogen activators such as t-PA may reflect diminished stimulation of synthesis of PAI-1.

Acknowledgments

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